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Chronic Stress Impairs Collateral Blood Flow Recovery in Aged Mice

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Abstract Chronic stress is associated with increased risk of cardiovascular diseases. Aging is also associated with vascular dysfunction. We hypothesize that chronic stress accelerates collateral dysfunction in old mice. Mice were subjected to either chronic social defeat (CSD) or chronic cold stress (CCS). The CSD mice were housed in a box inside an aggressor's cage and exposed to the aggressor. The CCS group was placed in iced water. After chronic stress, mice underwent femoral artery ligation (FAL) and flow recovery was measured. For the CSD group, appearance and use scores of the foot and a behavioral test were performed. CSD impaired collateral flow recovery after FAL. Further, stressed mice had greater ischemic damage, impaired foot function, and altered behavior. The CCS mice also showed impaired collateral flow recovery. Chronic stress causes hind limb collateral dysfunction in old mice, a conclusion reinforced by the fact that two types of stress produced similar changes.

Keywords Chronic stress · Blood flow · Collaterals · Hind limb · Femoral artery ligation · Flow recovery · Ischemia · Mouse

Abbreviations

CSD	Chronic social defeat stress
Ctrl CSD	Control chronic social defeat stress
CCS	Chronic cold stress
Ctrl CCS	Control chronic cold stress
AGG	Aggressor
FAL	Femoral artery ligation
LDPI	Laser Doppler perfusion imaging
PTSD	Posttraumatic stress disorder

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Introduction

Chronic stress has been identified as one of the major risk factors for cardiovascular morbidity and mortality [1–5]. In humans, stress contributes to endothelial dysfunction [4] and increases both atherosclerotic plaque development [5] and the incidence of acute myocardial infarction [6–8]. In rats, endothelial dysfunction results in endothelium-dependent increased sensitivity to phenylephrine and decreased relaxation in response to acetylcholine [9], both of which have been taken as findings that convey an increased propensity to atherogenesis. In this regard, chronic stress in mice, when sufficient to result in depressive symptoms, is associated not only with endothelial dysfunction [4] but also with peripheral vascular disease [10].

Several studies have also identified that chronic stress increases ischemic tissue damage following occlusion of the artery supplying the tissue. Balkaya et al. showed that mice subjected to chronic stress had significant increases in brain ischemic lesion size following occlusion of the middle cerebral artery [11]. Ritchie et al. demonstrated that rats subjected to chronic mild stress after permanent bilateral occlusion of the carotid arteries had significantly greater loss of hippocampus pyramidal cells compared to non-stressed controls [12]. Further, Wu et al. reported that rats with chronic mild stress had a greater incidence of ischemia-induced ventricular tachyarrhythmias during acute myocardial infarction [13].

There are also interesting interactions between aging and several of the above atherogenic-related mechanisms. Studies

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14. ABSTRACT
Chronic stress is associated with increased risk of cardiovascular diseases. Aging is also associated with vascu- lar dysfunction. We hypothesize that chronic stress accelerates collateral dysfunction in old mice. Mice were subjected to either chronic social defeat (CSD) or chronic cold stress (CCS). The CSD mice were housed in a box inside an aggres- sor???s cage and exposed to the aggressor. The CCS group was placed in iced water. After chronic stress, mice underwent femoral artery ligation (FAL) and flow recovery was mea- sured. For the CSD group, appearance and use scores of the foot and a behavioral test were performed. CSD impaired collateral flow recovery after FAL. Further, stressed mice had greater ischemic damage, impaired foot function, and altered behavior. The CCS mice also showed impaired collat- eral flow recovery. Chronic stress causes hind limb collateral dysfunction in old mice, a conclusion reinforced by the fact that two types of stress produced similar changes.

15. SUBJECT TERMS
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have demonstrated that oxidative stress increases with aging [14, 15], and individuals who experience chronic stress present with higher risk for earlier development of chronic diseases that typically occur consequent to aging. Moreover, chronic psychological stress is associated, in leukocytes, with shorter telomere length and lower telomerase activity [16], both of which are biomarkers of age [17]. Simon et al. also observed telomere shortening in patients with mood disorders, which was estimated to be equivalent to 10 years of aging [18]. Recent studies have highlighted that age-associated diseases (including cardiovascular-related) are influenced by proinflammatory cytokines. Cytokine IL-6, which is affected by chronic stress, plays a central role in promoting the production of C-reactive protein (CRP), a risk factor associated with atherosclerosis [19, 20]. Chronic stress, mental disorders, and non-pathological psychological stress states are associated with molecular, cellular, and clinical signs of accelerated aging. Moreover, somatic symptoms of depression, such as loss of energy and trouble sleeping, are associated with vascular dysfunction [21]; however, depression, as a cause of vascular dysfunction, remains a matter of debate [22, 23].

Recently, we found in mice that aging is associated with an actual decrease in the number of collaterals and with their impaired function [24]. We ascertained the biological importance of these changes by demonstrating that the aging-related impaired collateral function was associated with worse ischemic tissue injury following vascular occlusion. Attenuation of collateral function also has a major impact on clinical outcomes in human vascular occlusive disease [25].

Based on our focus on the effects of aging on the collateral circulation and on the effects of stress on biological functions, in the present investigation, we examined whether stress, like aging, can lead to collateral dysfunction or—seen from a slightly different perspective—whether stress accelerates the impaired collateral function already set in motion by the aging process. To this end, we studied the effects of chronic social stress on collateral flow recovery after hind limb ischemia in mice in advanced middle age (20 months old) when collateral dysfunction is already demonstrable [24]. We also used chronic physical/neurogenic stress to determine if the potential abnormalities caused by chronic social stress in collateral function were specific to this type of stress or can occur in other forms of stress.

Methods

Animals

Male, 20-month-old C57BL/6 mice were obtained from the National Institute of Aging. All experiments were conducted according to NIH guidelines and were approved by the MedStar Health Research Institute Animal Care and Use Committee. For the chronic social stress study, mice were divided into two groups: chronic social defeat (CSD) (n=10) and Ctrl CSD (n=8). For the CSD group, mice were housed in a small wire box placed inside an aggressor's home cage for 6 h per day. Mice were randomly exposed to aggressor (AGG) three times per day for 1 min or up to ten attacks or bleeding [26]. This protocol was performed for 20 days (uninterrupted). Control animals for the CSD group were housed for 6 h in a small wire box in their own home cage in a similar food/liquid-deprived environment in a separate room from the AGGs.

Sixteen additional mice were divided into two groups to study the chronic physical/neurogenic stress: chronic cold stress (CCS) and controls (n=8 in each group). The CCS group of mice was subjected to 4 weeks (5 days per week) of placement in a cage containing 1 cm of iced water for 1 h per day. The age-matched Ctrl CCS group was not stressed [27, 28]. Figure 1 shows a summary of the timeline of the experiments.

Hind limb Ischemia

Femoral artery ligation (FAL) was performed as described [24]. Mice were anesthetized with 1.25 % isoflurane/O_2 and the hind limbs were depilated. Body temperature was maintained at 37.0±0.5 °C. The left femoral artery was exposed through a 2-mm incision without retraction and with minimal tissue disturbance. A 7–0 ligature was placed distal to the origin of the lateral caudal femoral and superficial epigastric arteries (the latter was also ligated) and proximal to the genu artery. The femoral artery was transected between the sutures and separated 1–2 mm. The wound was irrigated with saline and closed, and one dose of analgesia (0.05–0.1 mg/kg) buprenorphine was administered.

LDPI

Laser Doppler perfusion imaging (LDPI) was used to record serial blood flow measurement pre-operatively, immediately post-operatively, and over the course of the subsequent 4 weeks at 7, 14, 21, and 28 days. Excessive hair was removed from the limb before imaging, and the mice were placed on a heating pad at 37 °C to minimize temperature variation.

Muscle Function and Ischemia At 5 and 14 days post-FAL, the animals were evaluated for right hind limb appearance (index of ischemia): 0, normal; 1–5, cyanosis or loss of nail(s), where the score is dependent on the number of nails affected; 6–10, partial or complete atrophy of digit(s), where the score reflects the number of digits affected; and 11, partial atrophy of forefoot [21]. Hind limb use scores (index of muscle function) were obtained: 0, normal; 1, no toe flexion; 2, no plantar flexion; and 3, dragging foot [24].

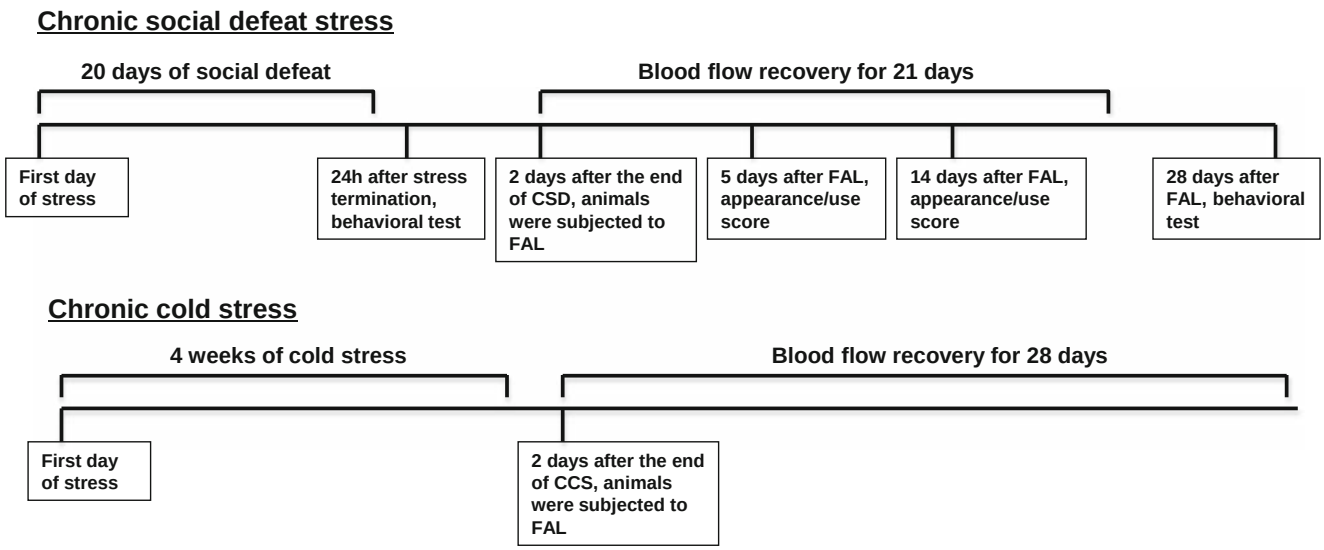

Fig. 1 Time line of study. For the CSD group, animals received the social defeat for 20 days and behavioral tests were performed 24 h after the last day of stress. Two days after stress protocol termination, mice (CSD and CSD controls) were subjected to hind limb ischemia and blood flow recovery was measured every week until day 21. On day 28, behavioral test was performed again. For CCS groups, animals were stressed in iced water for 28 days, and 2 days after the stress protocol termination, mice were subjected to hind limb ischemia and blood flow recovery was measured every week until day 2

Behavioral Partition Test (Freezing, Grooming, Partition Avoidance, and Tail Rattling)

We performed behavioral evaluations 1 day before and after the CSD protocol. The aggressor home cage (48×27×20 cm) was bisected with a plastic fenestrated partition (1 cm^2 holes) that permits the passage of sensory cues but prevents direct physical contact. The subject mouse was placed on the opposite side of the partition from the aggressor and video recorded for the entire 5 min test. The videos were analyzed with Ethovision XT v.7 software (Noldus®, Leesburg, VA, USA) using 15 samples per second, dynamic subtraction detection, object always darker than background, erosion and dilation filters of one pixel, and one sample interval for averaging filter. Behavioral actions such as freezing, grooming, and avoidance were analyzed and measured. Grooming duration was considered as the total time spent licking the paws or washing the nose, face, and other body parts. Tail rattling was observed as rapid vibration of the tail or vigorous tapping of the tail on the floor when facing the partition [26].

Statistics

For the continuous outcome variable for blood flow recovery, the differences in the means at each time point between the experimental groups were tested using two-sample *t* tests and the non-parametric Wilcoxon Rank sum test. Repeated measures ANOVA models (mixed models) were specified to examine the changes in the means over time for each time point (comparing days 7, 14, and 21 for social stress and comparing days 7, 14, and 21 for chronic stress) and to examine experimental groups for the outcome variable flows of social stress and cold stress by including dummy variables for time points and the experimental groups. Similar models were run, adding interaction terms between time points and the experimental model group in the models to allow for different slopes over time for each experimental group.

Differences in means for appearance/use score were analyzed by Student's *t* test or one-way ANOVA with Bonferroni correction. Probability values of less than 0.05 were considered significant.

Results

Chronic Social Defeat Stress

Hind limb Blood Flow Recovery After Femoral Artery Ligation

Immediately after ligation of the femoral artery (day 0), the CSD and control groups had similar blood flow. However, collateral flow recovery over time was significantly impaired in the stressed group (Fig. 2).

Appearance and Use Score

Biological relevance of the impaired collateral-dependent blood flow recovery was demonstrated by ischemia-related damage to hind limb function. The CSD group demonstrated higher appearance score at 5 and 14 days after FAL (trend).

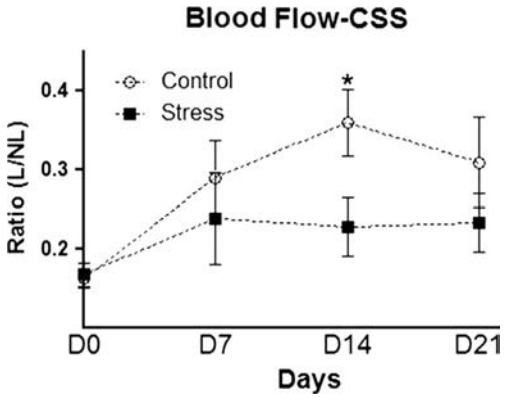

Fig. 2 Blood flow recovery measurement after FAL. Blood flow measured for control (*open circle*) and stressed mice (*closed square*) (n=10 stress and n=8 control) depicting mean±SEM for each day points. In CSD, the blood flow significantly increases over time in the control mice (*p<0.01), a change not seen in the stressed mice; overall, there is a significant difference between the longitudinal trajectories navigated by control vs. stressed mice (*p<0.04). The ratio between ligated and non-ligated legs (L/NL) was used to avoid the variation related to the mouse core temperature; this parameter is well established in blood flow analysis

The use score was significantly higher 5 days after FAL and showed a higher trend at 14 days (Fig. 3).

Behavioral Test

The behavioral actions of the CSD and control groups were measured before and after the stress protocol. Avoidance of stressor (fear response) was measured as the (inverse of) time spent per visit to partition. CSD mice spent significantly less time visiting to partition than controls day 1 and 4 weeks after

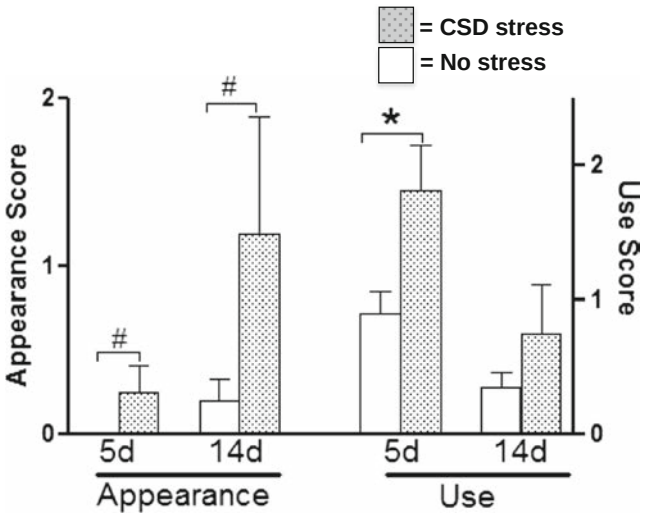

Fig. 3 Appearance score and use score. CSD control (*white bar*) and stressed (*shaded bar*) mice are plotted against the *left and right Y*-axis, respectively depicting mean±SEM. Welch's t test measures the differences, *p<0.05. Appearance score showed a strong increased trend ($^{\#}$0.1 <p<0.05) in stressed mice, while there is a significant increment of use score among the stressed mice on day 5 (*p<0.05), but not significant on day 14

the stress (Fig. 4a, p<0.05). Freezing (fear response) was measured as the immobility threshold that was set at >20 % repositional shift of the mouse contour during each frame (1/15 s) of the video recording. CSD mice froze significantly more times than controls 1 day after the stress (p<0.05); however, after 4 weeks, there is no difference between the groups (Fig. 4b). Grooming (anxiogenic marker) was measured by manual inspection. CSD mice groomed significantly more than controls on day 1 after the stress (p<0.05), but not 4 weeks after stress (Fig. 4c).

Chronic Cold Stress

Hind limb Blood Flow Recovery After Femoral Artery Ligation CCS Group

Chronic cold stress yielded similar blood flow recovery results after FAL as we observed in the chronic social defeat stress group. Immediately after ligation of the femoral artery (day 0), blood flow was no different between the CCS and control groups. However, impaired flow recovery was observed on day 7 in the CCS group and throughout the period of observation (day 28; Fig. 5).

Discussion

Different Types of Chronic Stress Cause Collateral Dysfunction in Old Mice

In this study, we investigated whether chronic stress accelerates the collateral dysfunction associated with aging. To accomplish this, we assessed the effects of stress on hind limb collateral function in mice at an age (20 months) at which age-induced collateral dysfunction is already beginning to appear [24]. For stress interventions, we first used a model of psychosocial chronic stress and then determined whether the resulting collateral dysfunction was limited to this specific type of stress by studying another model of chronic stress—physical/neurogenic—and found similar results.

For psychosocial stress, we used a chronic social defeat (or aggressor stress) model, subjecting the animals to multiple stressor exposures to model the stress of unpredictable threats [26]. This chronic social defeat stress model has been shown to induce physiological changes such as sympathetic nerve stimulation, metabolic alterations in the heart and brain, and cardiac fibrosis and, in many ways, provides a posttraumatic stress disorder (PTSD) model for mice [26]. As a model of physical/neurogenic stress, we used the cold stress protocol previously reported [27, 28].

Both models of stress led to impaired collateral flow recovery following femoral artery occlusion (Figs. 2 and 5).

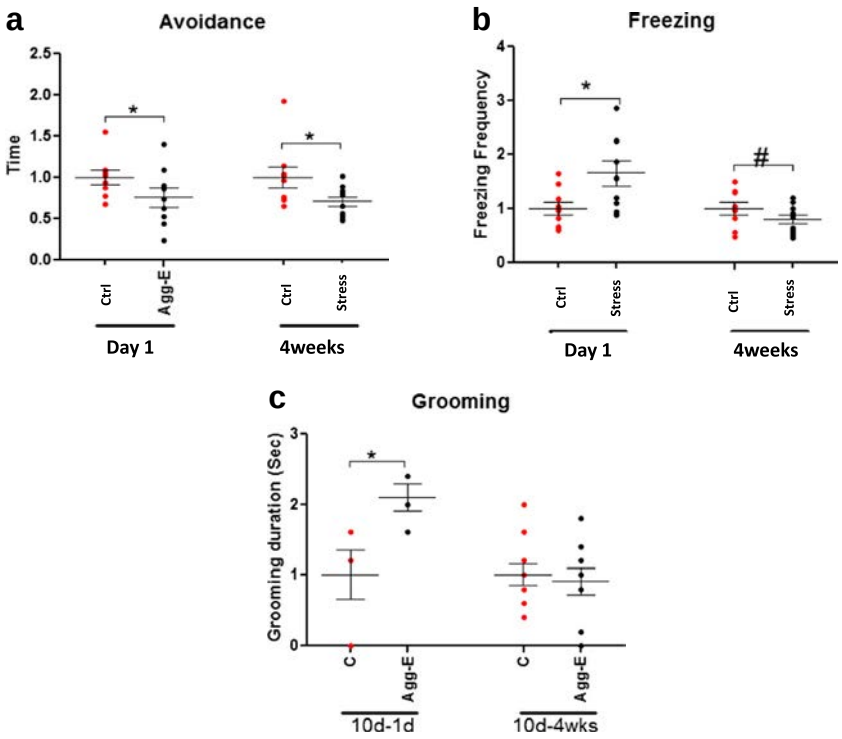

Fig. 4 Partition test-behavior aspects 1 day and 4 weeks after CSD protocol. **a** Stressed mice showed greater avoidance of the area close to the aggressor mice (*$p<0.05$) in both time points. **b** Stressed mice demonstrated greater freezing behavior on day 1 after the stress protocol (*$p<0.05$), but no difference at 4 weeks after the protocol termination ($^{\#}0.1<p<0.05$). **c** Stressed mice spent significantly more time grooming than controls on day 1 after the stress (*$p<0.05$), but not 4 weeks after stress

Although flow measured immediately after FAL was unaffected by either type of chronic stress, over the succeeding days and weeks, the normal increase in blood flow that marks recovery from FAL was significantly impaired in the stressed mice.

We had previously demonstrated in 20- and 30-month-old mice [24] that collateral dysfunction progresses over this time. Thus, the present results demonstrate that chronic stress accelerates aging-associated hind limb collateral dysfunction in 20-month-old C57BL/6 mice following femoral artery ligation and does so for up to 4 weeks following termination of the stress stimulus.

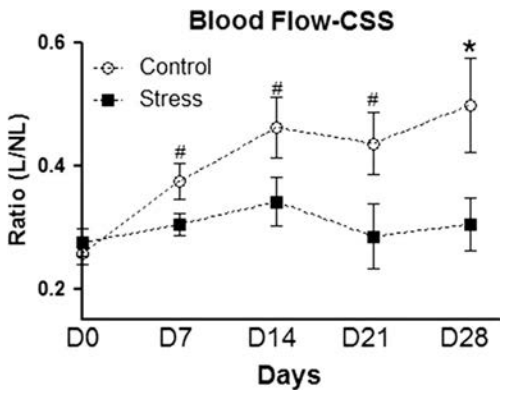

Fig. 5 Blood flow recovery measurement after FAL/CCS. The CCS group showed significantly impaired blood flow recovery compared to control, which was similar to the results in the CSD group (Fig. 2). Blood flow significantly increases in the control mice over time ($^{\#}p<0.05$) and marginally increases at 7, 14, and 21 days ($n=8$ each group). Overall, there is a significant difference between the longitudinal trajectories of navigated by control and CCS mice (*$p<0.01$)

Many publications [4, 8–10] have shown that stress has a negative impact on the vasculature. However, as best we can ascertain, this is the first study to report deleterious effects of chronic stress on collateral function. Importantly, we found that the response to stress is not related to a specific type of stress, as we observed that two different types of chronic stress exerted a similar impact on collateral function (Figs. 2 and 5).

The impaired collateral flow recovery could be explained by a decrease in collateral number, impaired collateral remodeling, or alterations in vasoreactivity of the collaterals. Although this study was not designed to identify the precise mechanisms contributing to the stress-induced collateral dysfunction, if it were caused by a decrease in collateral number, we would have expected that the stressed mice would have had a lower collateral flow immediately after femoral artery occlusion. This, however, was not observed. The collateral dysfunction observed in this study might be due to an impairment in eNOS/NO signaling pathway. It is well established that chronic stress increases oxidative stress [29, 30], which causes uncoupling of eNOS, leading to blunted vascular relaxation and a further increase in oxidative stress in the vascular environment [31].

CSD Causes Impairment of Foot Function and Ischemic Damage After FAL

To demonstrate the functional relevance of the impaired collateral blood flow recovery, we performed a blinded evaluation of hind limb function and appearance. We found that the

functional capacity of the ischemic hind limb of the CSD group was compromised compared to its controls (Fig. 3). Moreover, the ischemic tissue damage score showed a strong trend of worsened damage in the stressed group (Fig. 3).

CSD Causes Altered Behavior

In addition to the effects on the collateral circulation, the impact of the chronic social defeat stress also had the expected behavioral effects, indicating the broad systemic effects of this intervention. The behavioral response of the mice subjected to CSD was measured by the partition test 1 day and 4 weeks after stress protocol termination (Fig. 4). The anxiogenic response to aggressor mice (grooming) was higher on day 1 after CSD protocol completion; this effect waned and disappeared by 4 weeks. The fear response (avoidance of stressor and freezing) was greater in CSD vs. control mice; this effect persisted through the entire 4 weeks that were measured after the last aggressor exposure (CSD mice avoided the aggressor mice 4 weeks after the stress protocol; however, the freezing behavior did not persist after 4 weeks). These results are compatible with prior studies demonstrating the behavioral effects of CSD stress and further indicate that some altered CSD-induced behaviors persist after home cage rest for up to 4 weeks.

Conclusions

The results of this investigation demonstrate that chronic stress exerts long-term deleterious effects on collateral flow recovery following occlusion of a major artery. It is likely, although still to be proven, that stress may cause similar compromise of collateral function in humans, which, if so, has major clinical implications. This has particular resonance in patients suffering from PTSD, co-morbid depression, and anxiety disorders, as the general categories of behavioral alterations we observed in our mice are not dissimilar to those experienced by such patients. Future work will explore the molecular mechanisms in more detail by which stress causes collateral dysfunction.

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Animal Studies Research was conducted in compliance with the Animal Welfare Act and other Federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the Guide for the Care and Use of Laboratory Animals (NRC 2011) in facilities that are fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care, International.

References

1. Rozanski, A., Blumenthal, J. A., & Kaplan, J. (1999). Impact of psychological factors on the pathogenesis of cardiovascular disease and implications for therapy. *Circulation, 99*(16), 2192–2217.
2. Everson-Rose, S. A., & Lewis, T. T. (2005). Psychosocial factors and cardiovascular diseases. *Annual Review of Public Health, 26*, 469–500.
3. Kumari, M., Grahame-Clarke, C., Shanks, N., et al. (2003). Chronic stress accelerates atherosclerosis in the apolipoprotein E deficient mouse. *Stress, 6*(4), 297–299.
4. McDermott, M. M., Greenland, P., Guralnik, J. M., et al. (2003). Depressive symptoms and lower extremity functioning in men and women with peripheral arterial disease. *Journal General and International Medication, 18*(6), 461–467.
5. Yan, L. L., Liu, K., Matthews, K. A., et al. (2003). Psychosocial factors and risk of hypertension: the coronary artery risk development in young adults (CARDIA) study. *JAMA, 290*(16), 2138–2148.
6. Rosengren, A., Hawken, S., Ounpuu, S., Investigators, I. N. T. E. R. H. E. A. R. T., et al. (2004). Association of psychosocial risk factors with risk of acute myocardial infarction in 11,119 cases and 13,648 controls from 52 countries (the INTERHEART study): case–control study. *Lancet, 364*, 953–962.
7. Roepke, S. K., Allison, M., Von Känel, R., et al. (2012). Relationship between chronic stress and carotid intima-media thickness (IMT) in elderly Alzheimer's disease caregivers. *Stress, 15*(2), 121–9.
8. Vitaliano, P. P., Scanlan, J. M., Zhang, J., et al. (2002). A path model of chronic stress, the metabolic syndrome, and coronary heart disease. *Psychosomatic Medicine, 64*(3), 418–435.
9. Harrison, D. G., Gongora, M. C., Guzik, T. J., et al. (2007). Oxidative stress and hypertension. *Journal of American Social Hypertension, 1*, 30–44.
10. Harrison, D., Griendling, K. K., Landmesser, U., et al. (2003). Role of oxidative stress in atherosclerosis. *American of Journal in Cardiology, 91*, 7A–11A.
11. Balkaya, M., Prinz, V., Custodis, F., et al. (2011). Stress worsens endothelial function and ischemic stroke via glucocorticoids. *Stroke, 42*, 3258–3264.
12. Ritchie, L. J., De Butte, M., & Pappas, B. A. (2004). Chronic mild stress exacerbates the effects of permanent bilateral common carotid artery occlusion on CA1 neurons. *Brain Research, 1014*(1–2), 228–235.
13. Wu, W., Li, Y., Lu, Z., et al. (2012). Increased susceptibility to ischemia-induced ventricular tachyarrhythmias in depressed rats: involvement of reduction of connexin 43. *Experimental Therapy and Medication, 3*(2), 192–194.
14. Liu, D., & Xu, Y. (2011). p53, oxidative stress, and aging. *Antioxidants and Redox Signaling, 15*(6), 1669–1678.
15. Finkel, T., & Holbrook, N. J. (2000). Oxidants, oxidative stress and the biology of ageing. *Nature, 408*, 239–247.

16. Richter, T., & von Zglinicki, T. (2007). A continuous correlation between oxidative stress and telomere shortening in fibroblasts. *Experimental Gerontology, 42*, 1039–1042.
17. Maynard, S., Schurman, S. H., Harboe, C., et al. (2009). Base excision repair of oxidative DNA damage and association with cancer and aging. *Carcinogenesis, 30*, 2–10.
18. Simon, N. M., Smoller, J. W., McNamara, K. L., et al. (2006). Telomere shortening and mood disorders: preliminary support for a chronic stress model of accelerated aging. *Biological Psychiatry, 60*, 432–435.
19. Papanicolaou, D. A., Wilder, R. L., Manolagas, S. C., et al. (1998). The pathophysiologic roles of interleukin-6 in human disease. *Annals of Internal Medicine, 128*(2), 127–137.
20. Kiechl, S., Egger, G., Mayr, M., et al. (2001). Chronic infections and the risk of carotid atherosclerosis: prospective results from a large population study. *Circulation, 103*(8), 1064–1070.
21. Michal, M., Wiltink, J., Kirschner, Y., et al. (2013). Differential associations of depressive symptom dimensions with cardiovascular disease in the community: results from the Gutenberg health study. *PLoS ONE, 8*(8), e72014.
22. Frasure-Smith, N., & Lesperance, F. (2009). Depression and cardiac risk: present status and future directions. *Heart, 96*, 173–176.
23. Ormel, J., & de Jonge, P. (2011). Unipolar depression and the progression of coronary artery disease: toward an integrative model. *Psychotherapy and Psychosomatics, 80*, 264–274.
24. Faber, J. E., Zhang, H., Lassance-Soares, R. M., et al. (2011). Aging causes collateral rarefaction and increased severity of ischemic injury in multiple tissues. *Arteriosclerosis, Thrombosis, and Vascular Biology, 31*(8), 1748–1756.
25. Meier, P., Gloekler, S., Zbinden, R., et al. (2007). Beneficial effect of recruitable collaterals: a 10-year follow-up study in patients with stable coronary artery disease undergoing quantitative collateral measurements. *Circulation, 116*(9), 975–983.
26. Hammamieh, R., Chakraborty, N., De Lima, T. C., et al. (2012). Murine model of repeated exposures to conspecific trained aggressors simulates features of post-traumatic stress disorder. *Behavioural Brain Research, 235*(1), 55–66.
27. Li, L., Jonsson-Rylander, A. C., Abe, K., et al. (2005). Chronic stress induces rapid occlusion of angioplasty-injured rat carotid artery by activating neuropeptide Y and its Y1 receptors. *Arteriosclerosis, Thrombosis, and Vascular Biology, 25*(10), 2075–2080.
28. Najafi, A. H., Aghili, N., Tilan, J. U., et al. (2013). A new murine model of stress-induced complex atherosclerotic lesions. *Disease Models & Mechanisms, 6*(2), 323–331.
29. Epel, E. S., Blackburn, E. H., Lin, J., et al. (2004). Accelerated telomere shortening in response to life stress. *Proceedings of the National Academy of Sciences of the United States of America, 101*, 17312–17315.
30. Aschbacher, K., O'Donovan, A., Wolkowitz, O. M., et al. (2013). Good stress, bad stress and oxidative stress: insights from anticipatory cortisol reactivity. *Psychoneuroendocrinology, 38*, 1698–1708.
31. Epstein, S. E., Lassance-Soares, R. M., Faber, J. E., et al. (2012). Effects of aging on the collateral circulation, and therapeutic implications. *Circulation, 125*(25), 3211–3219.